

**Online Appendix 2. PCR primers.****Primers for modern DNA:**

| Marker                        | Fragment                                | For primer                  |
|-------------------------------|---|-----------------------------|
| <i>Col/II</i>                 | barcode region (LCO-HCO)                | GGTCAACAAATCATAAAGATATTGG   |
|                               | second part (Pat-Jerry)                 | CAACAYTTATTTTGATTTTTTGG     |
|                               | third part (C1-J-2783/C2-N-3812)        | TAGGATTAGCTGGAATACC         |
| <i>16S</i>                    |   | CCCGCCTGTTTATCAAAAACAT      |
| <i>Aact</i>                   |   | TGATTTTAAGCTGCACAAGGA       |
| <i>ArgK</i>                   |   | TNACYGARKCCARTAYAAG         |
| <i>Cad</i>                    | first half                              | GGNGTNACNACNGCNTGYTTYGARCC  |
|                               | second half                             | KGGATTYTCNGAYAAACAAATNGC    |
| <i>Cat</i>                    |   | TCAAGACTGCGATTCAAACA        |
| <i>Cmdh</i>                   | first half                              | GAYATNGCNCCNATGATGGGNGT     |
|                               | second half                             | GCNCCNTCWATNCCNAAAGA        |
|                               | <i>Heliconius</i> -specific second half | YAAAGTDGCHCGBAAGGATG        |
| <i>Ddc</i>                    |   | TGGYTICAYGTIGAYGCNGCNTAYGC  |
| <i>Dpp</i>                    | (dpp-f34/dpp-r327)                      | AGAGAACGTGGCGAGACACTG       |
| <i>EF1<math>\alpha</math></i> | first half (Starsky-Monica)             | CACATYAACATTGTCGTSATYGG     |
|                               | second half (AlF-EFrcM4)                | GAGGAAATYAARAARGAAG         |
| <i>gapdh</i>                  |   | AARGCTGGRGCTGAATATGT        |
|                               | <i>Heliconius</i> -specific             | GAWGGKGGTG CYAAGAARGT       |
| <i>GlyRS</i>                  |   | AGACACGTTTTAACATT           |
| <i>Hcl</i>                    |   | GCCGTAAAAGCAACCAC           |
| <i>Hsp40</i>                  |   | CACAATGGTCCAGGCGCT          |
| <i>Idh</i>                    |   | GGWGAYGARATGACNAGRATHATHTGG |
|                               | <i>Heliconius</i> -specific             | TTTRGGRATGGAAWATCGTG        |
| <i>Lm</i>                     |   | TCCAGAATGCTATGCTTGTG        |
| <i>Rps2</i>                   |   | ATCWCGYGGTGGYGATAGAG        |
| <i>Rps5</i>                   |   | ATGGCNGARGARAAYTGGAAYGA     |
| <i>Tada3</i>                  |   | GTGGAATGGGAGGAAGTGT         |
| <i>Trh</i>                    |   | GATGCCACGTCCGTTAGAGA        |
| <i>Vas</i>                    |   | ATCACAAGACTTCTCCGTTT        |
| <i>Wg</i>                     |   | GARTGYAARTGYCAYGGYATGTCTGG  |

**Primers for historical DNA:**

|                               |            |                                 |
|-------------------------------|------------|---------------------------------|
| <i>Col/II</i>                 | Ron-Nancy  | GGA GCY CCW GAT ATA GCT TTC CC  |
|                               | Brian-Mila | CTT CTA TAT TAT GAA GAT TAG G   |
|                               | Jerry-Mila | C AAC AYT TAT TTT GAT TTT TTG G |
|                               | Brian-Pat  |                                 |
| <i>EF1<math>\alpha</math></i> | part1      | CACATYAACATTGTCGTSATYGG         |
|                               | part3      | TcAAgAACATGATcACyGG             |

# Sheet2

| Rev primer                  | Tm (°C) | Reference               |
|-----------------------------|---------|-------------------------|
| TAAACTTCAGGGTGACCAAAAAATCA  | 50      | Wahlberg et al. 2009    |
| ATCCATTACATATAATCTGCCATA    | 50      | Wahlberg et al. 2010    |
| CATTAGAAGTAATTGCTAATTTACTA  | 52      | Beltran et al. 2007     |
| CCCTCCGGTTTGAACCTCAGATC     | 50      | Beltran et al. 2007     |
| ACTTACAATTTTTCAATCAT        | 53      | Salazar et al. 2010     |
| TTGATSAGYTCRGCGATG          | 55      | Wahlberg and Wheat 2008 |
| CATTGWGCKGCWACTGTATC        | 56      | Wahlberg and Wheat 2008 |
| TTRTTNGGNARYTGNCCNCCCAT     | 56      | Wahlberg and Wheat 2008 |
| TGTCTTCAGTTTGTCCACT         | 51      | Salazar et al. 2010     |
| AAYTGNGTRGATGARTGRTTNCC     | 55      | Wahlberg and Wheat 2008 |
| AGNCCYTCNACDATYTTCCAYTT     | 50      | Wahlberg and Wheat 2008 |
| CCYAAGAACCARTCCCTCAT        | 50      |                         |
| CCCATNGTNACYTCYTC           | 48      | Wahlberg and Wheat 2008 |
| GAGGAAAGTTGCGTAGGAACG       | 58      | Beltran et al. 2007     |
| CATRTTGTCCKCGTGCCARCC       | 60      | Wahlberg and Wheat 2008 |
| ACAGCVACKGYTGYCTCATRTC      | 50      | Wahlberg and Wheat 2008 |
| GWTTGAATGTACTTGATRAGRTC     | 53      | Wahlberg and Wheat 2008 |
| GATMCCWGCAGCRGCATCAA        | 55      |                         |
| ATAGTGAATTCTCTAACTCT        | 53      | Salazar et al. 2010     |
| AACATATAAATTACACCAAA        | 48      | Salazar et al. 2010     |
| TCACTGCCTTCTCTCTTGAA        | 60      | Salazar et al. 2010     |
| TTYTTRCAIGCCCANACRAANCCNCC  | 55      | Wahlberg and Wheat 2008 |
| CCATRTCCTCRATYARCCTATG      | 50      |                         |
| TTAATTCAGATGTAAGCTCT        | 50      | Salazar et al. 2010     |
| ATGRGGCTTKCCRATCTTGT        | 52      | Wahlberg and Wheat 2008 |
| CGGTTRGAYTTRGCAACACG        | 55      | Wahlberg and Wheat 2008 |
| TTTGCGCGGGTAAATTTGTT        | 54      | Salazar et al. 2010     |
| CTATGAGCTTGTTGAATACC        | 50      | Salazar et al. 2010     |
| TTTTCTTCTTAAGTTACTGG        | 50      | Salazar et al. 2010     |
| ACTICGCARCAACCARTGGAATGTRCA | 52      | Beltran et al. 2007     |

|                                    |    |                      |
|------------------------------------|----|----------------------|
| CCT GGT AAA ATT AAA ATA TAA ACT TC | 50 | Wahlberg et al. 2009 |
| ATT AAT CCT GTA AAT AAW GG         | 50 | Wahlberg et al. 2009 |
|                                    | 53 | Wahlberg et al. 2009 |
| A TCC ATT ACA TAT AAT CTG CCA TA   | 53 | Wahlberg et al. 2009 |
| TrScgGTYTCGAaTTCCA                 | 58 |                      |
| GARGAyACTTCcTTcTTgA                | 51 |                      |